XVIII. "On the Reduction and Oxidation of the Colouring Matter of the Blood." By G. G. STOKES, M.A., Sec. R.S., Lucasian Professor of Mathematics in the University of Cambridge. Received June 16, 1864.

1. Some time ago my attention was called to a paper by Professor Hoppe *, in which he has pointed out the remarkable spectrum produced by the absorption of light by a very dilute solution of blood, and applied the observation to elucidate the chemical nature of the colouring matter. I had no sooner looked at the spectrum, than the extreme sharpness and beauty of the absorption-bands of blood excited a lively interest in my mind, and I proceeded to try the effect of various reagents. The observation is perfectly simple, since nothing more is required than to place the solution to be tried, which may be contained in a test-tube, behind a slit, and view it through a prism applied to the eye. In this way it is easy to verify Hoppe's statement, that the colouring matter (as may be presumed at least from the retention of its peculiar spectrum) is unaffected by alkaline carbonates and caustic ammonia, but is almost immediately decomposed by acids, and also, but more slowly, by caustic fixed alkalies, the coloured product of decomposition being the hæmatin of Lecanu, which is easily identified by its peculiar spectra. But it seemed to me to be a point of special interest to inquire whether we could imitate the change of colour of arterial into that of venous blood, on the supposition that it arises from reduction.

2. In my experiments I generally employed the blood of sheep or oxen obtained from a butcher; but Hoppe has shown that the blood of animals in general exhibits just the same bands. To obtain the colouring matter in true solution, and at the same time to get rid of a part of the associated matters, I generally allowed the blood to coagulate, cut the clot small, rinsed it well, and extracted it with water. This, however, is not essential, and blood merely diluted with a large quantity of water may be used; but in what follows it is to be understood that the watery extract is used unless the contrary be stated.

3. Since the colouring matter is changed by acids, we must employ reducing agents which are compatible with an alkaline solution. If to a solution of protosulphate of iron enough tartaric acid be added to prevent precipitation by alkalies, and a small quantity of the solution, previously rendered alkaline by either ammonia or carbonate of soda, be added to a solution of blood, the colour is almost instantly changed to a much more purple red as seen in small thicknesses, and a much darker red than before as seen in greater thickness. The change of colour, which recalls the difference between arterial and venous blood, is striking enough, but the change in the absorption spectrum is far more decisive. The two highly

* Virchow's Archiv, vol. xxiii. p. 446 (1862).

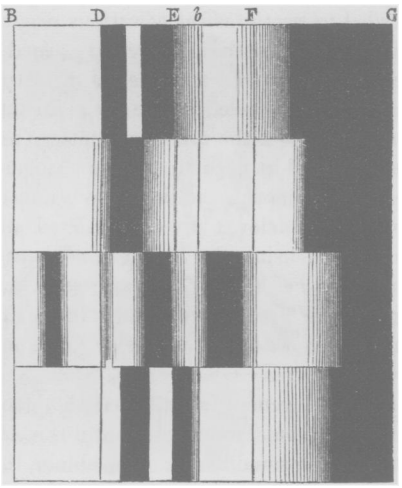
characteristic dark bands seen before are now replaced by a *single* band, somewhat broader and less sharply defined at its edges than either of the former, and occupying nearly the position of the bright band separating the dark bands of the original solution. The fluid is more transparent for the blue, and less so for the green than it was before. If the thickness be increased till the whole of the spectrum more refrangible than the red be on the point of disappearing, the last part to remain is *green*, a little beyond the fixed line *b*, in the case of the original solution, and *blue*, some way beyond F, in the case of the modified fluid. Figs. 1 and 2 in the accompanying woodcut represent the bands seen in these two solutions respectively.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.



4. If the purple solution be exposed to the air in a shallow vessel, it quickly returns to its original condition, showing the two characteristic bands the same as before; and this change takes place immediately, provided a small quantity only of the reducing agent were employed, when the solution is shaken up with air. If an additional quantity of the reagent be now added, the same effect is produced as at first, and the solution may thus be made to go through its changes any number of times.

5. The change produced by the action of the air (that is, of course, by the absorption of oxygen) may be seen in an instructive form on partly filling a test-tube with a solution of blood suitably diluted, mixing with a little of the reducing agent, and leaving the tube at rest for some time in a vertical position. The upper or oxidized portion of the solution is readily distinguished by its colour; and if the tube be now placed behind a slit and viewed through a prism, a dark band is seen, having the general form of a tuning-fork, like figs. 1 and 2, regarded now as a single figure, the line of separation being supposed removed.

6. Of course it is necessary to assure oneself that the single band in the green is not due to absorption produced merely by the reagent, as is readily done by direct observation of its spectrum, not to mention that in the region of the previous dark bands, or at least the outer portions of it, the solution is actually more transparent than before, which could not be occasioned by an *additional* absorption. Indeed the absorption due to the reagent itself in its different stages of oxidation, unless it be employed in most unnecessary excess, may almost be regarded as evanescent in comparison with the absorption due to the colouring matter; though if the solution be repeatedly put through its changes, the accumulation of the persalt of iron will presently tell on the colour, making it sensibly yellower than at first for small thicknesses of the solution.

7. That the change which the iron salt produces in the spectrum is due to a simple reduction of the colouring matter, and not to the formation of some compound of the colouring matter with the reagent, is shown by the fact that a variety of reducing agents of very different nature produce just the same effect. If protochloride of tin be substituted for protosulphate of iron in the experiment above described, the same changes take place as with the iron salt. The tin solution has the advantage of being colourless, and leaving the visible spectrum quite unaffected, both before and after oxidation, and accordingly of not interfering in the slightest degree with the optical examination of the solutions, but permitting them to be seen with exactly their true tints. The action of this reagent, however, takes some little time at ordinary temperatures, though it is very rapid if previously the solution be gently warmed. Hydrosulphate of ammonia again produces the same change, though a small fraction of the colouring matter is liable to undergo some different modification, as is shown by the occurrence of a slender band in the red, variable in its amount of development, which did not previously exist. In this case, as with the tin salt, the action is somewhat slow, requiring a few minutes unless it be assisted by gentle heat. Other reagents might be mentioned, but these will suffice.

8. We may infer from the facts above mentioned that *the colouring matter of blood,* like indigo, *is capable of existing in two states of oxidation, distinguishable by a difference of colour and a fundamental difference in the action on the spectrum. It may be made to pass from the more to the less oxidized state by the action of suitable reducing agents, and recovers its oxygen by absorption from the air.*

As the term *hæmatin* has been appropriated to a product of decomposition, some other name must be given to the original colouring matter. As it has not been named by Hoppe, I propose to call it *cruorine,* as suggested to me by Dr. Sharpey; and in its two states of oxidation it may conveniently be named *scarlet cruorine* and *purple cruorine* respectively, though the former is slightly purplish at a certain small thickness, and the latter is of a very red purple colour, becoming red at a moderate increase of thickness.

9. When the watery extract from blood-clots is left aside in a corked

bottle, or even in a tall narrow vessel open at the top, it presently changes in colour from a bright to a dark red, decidedly purple in small thicknesses. This change is perceived even before the solution has begun to stink in the least perceptible degree. The tint agrees with that of the purple cruorine obtained immediately by reducing agents; and if a little of the solution be sucked up from the bottom into a quill-tube drawn to a capillary point, and the tube be then placed behind a slit, so as to admit of analyzing the transmitted light without exposing the fluid to the air, the spectrum will be found to agree with that of purple cruorine. On shaking the solution with air it immediately becomes bright red, and now presents the optical characters of scarlet cruorine. It thus appears that scarlet cruorine is capable of being reduced by certain substances, derived from the blood, present in the solution, which must themselves be oxidized at its expense.

10. When the alkaline tartaric solution of protoxide of tin is added in moderate quantity to a solution of scarlet cruorine, the latter is presently reduced. If the solution is now shaken with air, the cruorine is almost instantly oxidized, as is shown by the colour of the solution and its spectrum by transmitted light. On standing for a little time, a couple of minutes or so, the cruorine is again reduced, and the solution may be made to go through these changes a great number of times, though not of course indefinitely, as the tin must at last become completely oxidized. It thus appears that purple cruorine absorbs *free* oxygen with much greater avidity than the tin solution, notwithstanding that the oxidized cruorine is itself reduced by the tin salt. I shall return to this experiment presently.

11. When a little acid, suppose acetic or tartaric acid, which does not produce a precipitate, is added to a solution of blood, the colour is quickly changed from red to brownish red, and in place of the original bands (fig. 1) we have a different system, nearly that of fig. 3. This system is highly characteristic; but in order to bring it out a larger quantity of substance is requisite than in the case of scarlet cruorine. The figure does not exactly correspond to any one thickness, for the bands in the blue are best seen while the band in the red is still rather narrow and ill-defined at its edges, while the narrow inconspicuous band in the yellow hardly comes out till the whole of the blue and violet, and a good part of the green, are absorbed. The difference in the spectra figs. 1 and 3 does not alone prove that the colouring matter is decomposed by the acid (though the fact that the change is not instantaneous favours that supposition), for the one solution is alkaline, though it may be only slightly so, while the other is acid, and the difference of spectra might be due merely to this circumstance. As the direct addition of either ammonia or carbonate of soda to the acid liquid causes a precipitate, it is requisite in the first instance to separate the colouring matter from the substance so precipitated.

This may be easily effected on a small scale by adding to the watery extract from blood-clots about an equal volume of ether, and then some glacial acetic acid, and gently mixing, but not violently shaking for fear

of forming an emulsion. When enough acetic acid has been added, the acid ether rises charged with nearly the whole of the colouring matter, while the substance which caused the precipitate remains in the acid watery layer below*. The acid ether solution shows in perfection the characteristic spectrum fig. 3. When most of the acid is washed out the substance falls, remaining in the ether near the common surface. If after removing the wash-water a solution, even a weak one, of ammonia or carbonate of soda be added, the colouring matter readily dissolves in the alkali. The spectrum of the transmitted light is quite different from that of scarlet cruorine, and by no means so remarkable. It presents a single band of absorption, very obscurely divided into two, the centre of which nearly coincides with the fixed line D, so that the band is decidedly less refrangible than the pair of bands of scarlet cruorine. The relative proportion of the two parts of the band is liable to vary. The presence of alcohol, perhaps even of dissolved ether, seems to favour the first part, and an excess of caustic alkali the second, the fluid at the same time becoming more decidedly dichroitic. The blue end of the spectrum is at the same time absorbed. The band of absorption is by no means so definite at its edges as those of scarlet cruorine, and a far larger quantity of the substance is required to develope it.

This difference of spectra shows that the colouring matter (hæmatin) obtained by acids is a product of the decomposition, or metamorphosis of some kind, of the original colouring matter.

When hæmatin is dissolved in alcohol containing acid, the spectrum nearly agrees with that represented in fig. 3.

12. Hæmatin is capable of reduction and oxidation like cruorine. If it be dissolved in a solution of ammonia or of carbonate of soda, and a little of the iron salt already mentioned, or else of hydrosulphate of ammonia, be added, a pair of very intense bands of absorption is immediately developed (fig. 4). These bands are situated at about the same distance apart as those of scarlet cruorine, and are no less sharp and distinctive. They are a little more refrangible, a clear though narrow interval intervening between the first of them and the line D. They differ much from the bands of cruorine in the relative strength of the first and second band. With cruorine the second band appears almost as soon as the first, on increasing the strength or thickness of the solution from zero onwards, and when both bands are well developed, the second band is decidedly broader than the first. With reduced hæmatin, on the other hand, the first band is already black and intense by the time the second begins to appear; then both bands increase, the first retaining its superiority until the two are on the

* If I may judge from the results obtained with the precipitate given by acetic acid and a neutral salt, a promising mode of separation of the proximate constituents of blood-crystals would be to dissolve the crystals in glacial acetic acid and add ether, which precipitates a white albuminous substance, leaving the hæmatin in solution.

point of merging into one by the absorption of the intervening bright band, when the two appear about equal.

Like cruorine, reduced hæmatin is oxidized by shaking up its solution with air. I have not yet obtained hæmatin in an acid solution in more than one form, that which gives the spectrum fig. 3, and which I have little doubt contains hæmatin in its oxidized form; for when it is withdrawn from acid ether by an alkali, I have not seen any traces of reduced hæmatin, even on taking some precautions against the absorption of oxygen. As the alkaline solution of ordinary hæmatin passes, with increase of thickness, through yellow, green, and brown to red, while that of reduced hæmatin is red throughout, the two kinds may be conveniently distinguished as *brown hæmatin* and *red hæmatin* respectively, the former or oxidized substance being the hæmatin of chemists.

13. Although the spectrum of scarlet cruorine is not affected by the addition to the solution of either ammonia or carbonate of soda, yet if after such addition the solution be either heated or alcohol be added, although there is no precipitation decomposition takes place. The coloured product of decomposition is brown hæmatin, as may be inferred from its spectrum. Since, however, the spectrum of an alkaline solution of brown hæmatin is only moderately distinctive, and is somewhat variable according to the nature of the solvent, it is well to add hydrosulphate of ammonia, which immediately developes the remarkable bands of red hæmatin. This is the easiest way to obtain them; but the less refrangible edge of the first band as obtained in this way is liable to be not quite clean, in consequence of the presence of a small quantity of cruorine which escaped decomposition.

Some very curious reactions are produced in a solution of cruorine by gallic acid combined with other reagents, but these require further study.

14. Hoppe proposed to employ the highly characteristic absorption-bands of scarlet cruorine in forensic inquiries. Since, however, cruorine is very easily decomposed, as by hot water, alcohol, weak acids, &c., the method would often be inapplicable. But as in such cases the coloured product of decomposition is hæmatin, which is a very stable substance, the absorption-bands of red hæmatin in alkaline solution, which in sharpness, distinctive character and sensibility rival those of scarlet cruorine itself, may be employed instead of the latter. The absorption-bands of brown hæmatin dissolved in a mixture of ether and acetic acid, or in acetic acid alone, are hardly less characteristic, but are not quite so sensitive, requiring a somewhat larger quantity of the substance.

15. I have purposely abstained from physiological speculations until I should have finished the chemico-optical part of the subject; but as the facts which have been adduced seem calculated to throw considerable light on the function of cruorine in the animal economy, I may perhaps be permitted to make a few remarks on this subject.

It has been a disputed point whether the oxygen introduced into the blood in its passage through the lungs is simply dissolved or is chemically

combined with some constituent of the blood. The latter and more natural view seems for a time to have given place to the former in consequence of the experiments of Magnus. But Liebig and others have since adduced arguments to show that the oxygen absorbed is, mainly at least, chemically combined, be it only in such a loose way, like a portion of the carbonic acid in bicarbonate of soda, that it is capable of being expelled by indifferent gases. It is known, too, that it is the red corpuscles in which the faculty of absorbing oxygen mainly resides.

Now it has been shown in this paper that we have in cruorine a substance capable of undergoing reduction and oxidation, more especially oxidation, so that if we may assume the presence of purple cruorine in venous blood, we have all that is necessary to account for the absorption and chemical combination of the inspired oxygen.

16. It is stated by Hoppe that venous as well as arterial blood shows the two bands which are characteristic of what has been called in this paper scarlet cruorine. As the precautions taken to prevent the absorption of oxygen are not mentioned, it seemed desirable to repeat the experiment, which Dr. Harley and Dr. Sharpey have kindly done. A pipette adapted to a syringe was filled with water which had been boiled and cooled without exposure to the air, and the point having been introduced into the jugular vein of a live dog, a little blood was drawn into the bulb. Without the water the blood would have been too dark for spectral analysis. The colour did not much differ from that of scarlet cruorine; certainly it was much nearer the scarlet than the purple substance. The spectrum showed the bands of scarlet cruorine.

This, however, does not by any means prove the absence of purple cruorine, but only shows that the colouring matter present was chiefly scarlet cruorine. Indeed the relative proportions of the two present in a mixture of them with one another and with colourless substances, can be better judged of by the tint than by the use of the prism. With the prism the extreme sharpness of the bands of scarlet cruorine is apt to mislead, and to induce the observer greatly to exaggerate the relative proportion of that substance.

Seeing then that the change of colour from arterial to venous blood *as far as it goes* is *in the direction* of the change from scarlet to purple cruorine, that scarlet cruorine is capable of reduction even in the cold by substances present in the blood (§ 9), and that the action of reducing agents upon it is greatly assisted by warmth (§ 7), we have every reason to believe that *a portion* of the cruorine present in venous blood exists in the state of purple cruorine, and is reoxidized in passing through the lungs.

17. That it is only a rather small proportion of the cruorine present in venous blood which exists in the state of purple cruorine under normal conditions of life and health, may be inferred, not only from the colour, but directly from the results of the most recent experiments *. Were it

* Funk's Lehrbuch der Physiologie, 1863, vol. i. § 108.

otherwise, any extensive hæmorrhage could hardly fail to be fatal, if, as there is reason to believe, cruorine be the substance on which the function of respiration mainly depends; nor could chlorotic persons exhale as much carbonic acid as healthy subjects, as is found to be the case.

But after death there is every reason to think that the process of reduction still goes on, especially in the case of warm-blooded animals, while the body is still warm. Hence the blood found in the veins of an animal some time after death can hardly be taken as a fair specimen as to colour of the venous blood in the living animal. Moreover the blood of an animal which has been subjected to abnormal conditions before death is of course liable to be altered thereby. The terms in which Lehmann has described the colour of the blood of frogs which had been slowly asphyxiated by being made to breathe a mixture of air and carbonic acid seem unmistakeably to point to purple cruorine*.

18. The effect of various indifferent reagents in changing the colour of defibrinated blood has been much studied, but not always with due regard to optical principles. The brightening of the colour, as seen by reflexion, produced by the first action of neutral salts, and the darkening caused by the addition of a little water, are, I conceive, easily explained; but I have not seen stated what I feel satisfied is the true explanation. In the former case the corpuscles lose water by exosmose, and become thereby more highly refractive, in consequence of which a more copious reflexion takes place at the common surface of the corpuscles and the surrounding fluid. In the latter case they gain water by endosmose, which makes their refractive power more nearly equal to that of the fluid in which they are contained, and the reflexion is consequently diminished. There is nothing in these cases to indicate any change in the mode in which light is absorbed by the colouring matter, although a change of tint to a certain extent, and not merely a change of intensity, may accompany the change of conditions under which the turbid mixture is seen, as I have elsewhere more fully explained†.

No doubt the form of the corpuscles is changed by the action of the reagents introduced; but to attribute the change of colour to this is, I apprehend, to mistake a concomitant for a cause, and to attribute, moreover, the change of colour to a cause inadequate to produce it.

19. Very different is the effect of carbonic acid. In this case the existence of a fundamental change in the mode of absorption cannot be questioned, especially when the fluid is squeezed thin between two glasses and viewed by transmitted light. I took two portions of defibrinated blood; to one I added a little of the reducing iron solution, and passed carbonic acid into the other, and then compared them. They were as nearly as possible alike. We must not attribute these apparently identical changes to two totally different causes if one will suffice. Now in the case of the iron

* Physiological Chemistry, vol. ii. p. 178.

† Philosophical Transactions, 1852, p. 527.

salt, the change of colour is plainly due to a deoxidation of the cruorine. On the other hand, Magnus removed as much as 10 or 12 per cent. by volume of oxygen from arterialized blood by shaking the blood with carbonic acid. If, as we have reason to believe, this oxygen was for the most part chemically combined, it follows that carbonic acid acts *as if it were* a reducing agent. We are led to regard the change of colour not as a *direct* effect of the *presence of carbonic acid*, but a consequence of the *removal of oxygen*. There is this difference between carbonic acid and the *real* reducing agents, that the former no longer acts on a dilute and comparatively pure solution of scarlet cruorine, while the latter act just as before.

If even in the case of blood exposed to an atmosphere of carbonic acid we are not to attribute the change of colour to the direct presence of the gas, much less should we attempt to account for the darker colour of venous than arterial blood by the small additional percentage of carbonic acid which the former contains. The ascertained properties of cruorine furnish us with a ready explanation, namely that it is due to a partial reduction of scarlet cruorine in supplying the wants of the system.

20. I am indebted to Dr. Akin for calling my attention to a very interesting pamphlet by A. Schmidt on the existence of ozone in the blood*. The author uses throughout the language of the ozone theory. If by ozone be meant the substance, be it allotropic oxygen or teroxide of hydrogen, which is formed by electric discharges in air, there is absolutely nothing to prove its existence in blood; for all attempts to obtain an oxidizing gas from blood failed. But if by ozone be merely meant oxygen in any such state, of combination or otherwise, as to be capable of producing certain oxidizing effects, such as turning guaiacum blue, the experiments of Schmidt have completely established its existence, and have connected it, too, with the colouring matter. Now in cruorine we have a substance admitting of easy oxidation and reduction; and connecting this with Schmidt's results, we may infer that scarlet cruorine is not merely a greedy absorber and a carrier of oxygen, but also an *oxidizing agent*, and that it is by its means that the substances which enter the blood from the food, setting aside those which are either assimilated or excreted by the kidneys, are reduced to the ultimate forms of carbonic acid and water, as if they had been burnt in oxygen.

21. In illustration of the functions of cruorine, I would refer, in conclusion, to the experiment mentioned in § 10. As the purple cruorine in the solution was oxidized almost instantaneously on being presented with free oxygen by shaking with air, while the tin-salt remained in an unoxidized state, so the purple cruorine of the veins is oxidized during the time, brief though it may be, during which it is exposed to air in the lungs, while the substances derived from the food may have little disposition to combine with free oxygen. As the scarlet cruorine is gradually reduced, oxidizing thereby a portion of the tin-salt, so part of the scarlet cruorine is gradually

* Ueber Ozon im Blute. Dorpat, 1862.

reduced in the course of the circulation, oxidizing a portion of the substances derived from the food or of the tissues. The purplish colour now assumed by the solution illustrates the tinge of venous blood, and a fresh shake represents a fresh passage through the lungs.

XIX. "Further Inquiries concerning the Laws and Operation of Electrical Force." By Sir W. Snow Harris, F.R.S., &c. Received June 8, 1864.

(Abstract.)

1. The author first endeavours to definitely express what is meant by *quantity of electricity*, *electrical charge*, and *intensity*.

By *quantity of electricity* he understands the actual amount of the unknown agency constituting electrical force, as represented by some arbitrary quantitative 'electrical' measure. By *electrical charge* he understands the quantity which can be sustained upon a given surface under a given electrometer indication. *Electrical intensity*, on the contrary, is 'the electrometer indication' answering to a given quantity upon a given surface.

2. The experiments of Le Monnier in 1746, of Cavendish in 1770, and the papers of Volta in 1779, are quoted as showing that bodies do not take up electricity in proportion to their surfaces. According to Volta, any plane surface extended in length sustains a greater charge,—a result which this distinguished philosopher attributes to the circumstance that the electrical particles are further apart upon the elongated surface, and consequently further without each other's influence.

3. The author here endeavours to show that, in extending a surface in length, we expose it to a larger amount of inductive action from surrounding matter, by which, on the principles of the condenser, the intensity of the accumulation is diminished, and the charge consequently increased; so that not only are we to take into account the influence of the particles on each other, but likewise their operation upon surrounding matter.

4. No very satisfactory experiments seem to have been instituted showing the relation of quantity to surface. The quantity upon a given surface has been often vaguely estimated without any regard to a constant electrometer indication or intensity. The author thinks we can scarcely infer from the beautiful experiment of Coulomb, in consequence of this omission, that the capacity of a circular plate of twice the diameter of a given sphere is twice the capacity of the sphere, and endeavours to show, in a future part of the paper (Experiment 16), that the charge of the sphere and plate are to each other not really as 1·2, but as $1:\sqrt{2}$, that is, as the square roots of the exposed surfaces; so that we cannot accumulate twice the quantity of electricity upon the plate under the same electrometer indication.

5. On a further investigation of the laws of electrical charge, the quantity which any plane rectangular surface can receive under a given intensity